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Review

Revisiting Poly(ADP-ribose) Polymerase as an Emerging Therapeutic Target for Various Cancer Treatments

Sunghark Kwon [1,2]

[1] Bright College, Hankyong National University, Anseong 17579, Republic of Korea; naritsuru@hknu.ac.kr
[2] Gyeonggi Regional Research Center, Hankyong National University, Anseong 17579, Republic of Korea

Abstract

Poly(adenosine diphosphate [ADP]-ribose) polymerases (PARPs) synthesize poly(ADP-robose) chains with linear or branched forms. PARPs play an essential role in the addition of ADP-ribose monomers to specific residues of proteins and are involved in repairing damaged DNA. In cancer therapy, particularly radiotherapy, PARP1 has emerged as a promising drug target. Moreover, PARP1 inhibition has been shown to sensitize cancer cells to mechanisms involving synthetic lethality. Consequently, the structure of PARP1 has been studied, and several PARP1 inhibitors have been developed and launched. Although the structure of the catalytic domain of PARP1 is known, the whole structure of PARP1 has not yet been elucidated. In addition, the precise mechanism underlying the function of PARP1 remains unclear. To develop novel anticancer agents targeting PARP1, current and comprehensive information on PARP1 is necessary. In this review, we describe the structure of PARP1 in complex with its inhibitors, as well as its native structure, which provides structural information on PARP1 inhibitor-binding modes. The pharmaceutical profiles of the launched PARP1 inhibitors are also summarized. In addition, we discuss possible strategies for the development of novel inhibitors targeting PARP1 based on the structural features of PARP1 and its inhibitor-binding modes.

Keywords: poly(adenosine diphosphate-ribose) polymerase; nicotine adenine dinucleotide; DNA repair; synthetic lethality; breast cancer; ovarian cancer; poly (adenosine diphosphate-ribose) polymerase inhibitor

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1. Introduction

Poly(adenosine diphosphate [ADP]-ribose) polymerases (PARPs) catalyze the synthesis of chains of poly(ADP-ribose) (PAR). PARPs transfer ADP-ribose monomers to several specific residues, such as glutamate, arginine, aspartate, serine, lysine, and cysteine, on their target proteins [1–3]. PARPs require oxidized nicotinamide adenine dinucleotide (NAD^+) as a substrate for polymerization and nicotinamide (NAM) is released from NAD^+ via a catalytic reaction. The formed PAR chains can be linear, branched, or mixed and range in length from tens nucleotides to as many as 200 [2,3].

The PARP family comprises 17 members (PARP1–4, -5a/b, and -6–16) [4]. PARP1–5 have PARP catalytic activity, whereas PARP6–8, -10–12, and -14–16 have mono(ADP-ribose) catalytic activity [4]. PARP9 and -13 do not have any catalytic activity [4]. Of these, PARP1–2 play vital roles in repairing single-stranded DNA breaks (SSBs) [5]. PARP1–2 detect SSBs and bind to break sites. This molecular event initiates the synthesis of PAR

chains, which recruit a group of proteins associated with various cellular responses, such as DNA repair, apoptosis, and telomere maintenance [2].

Much attention has been paid to PARP1 as a drug target in cancer therapy because of its involvement in SSBs. PARP1 is primarily associated with DNA repair of SSBs induced in cancer cells by radiotherapy [6]. Inhibition of PARP1 function can lead to irreversible DNA damage in cancer cells, followed by cell death. Thus PARP1 inhibitors can be used as anticancer agents in combination with radiotherapy. PARP inhibitors have attracted interest, since it was found that they function as single-agent chemotherapy, resulting in synthetic lethality of *BRCA*-mutant cancer cells [7–10].

Synthetic lethality is a genetic phenomenon in which simultaneous mutations in two genes lead to cell death, whereas a mutation in only one gene results in cell survival. BRCA1/2, a class of tumor suppressors in normal cells, is associated with homologous recombination [11]. Thus, BRCA1/2 are essential for DNA repair. However, if *BRCA1/2* genes are mutated and lose their intrinsic functions, cells have no choice but to depend only on PARP1/2 for DNA repair. Therefore, PARP inhibitors can be used as monotherapy targeting *BRCA*-mutant cancers.

Owing to the importance of PARP1 as a therapeutic target, several PARP1 inhibitors have been developed. These include olaparib [12,13], rucaparib [14,15], niraparib [16–18], and talazoparib [19,20]. The crystal structures of PARP1 in complex with its inhibitors, including its native form, have been determined using X-ray crystallography [21–26]. These structures provide valuable information regarding the inhibitor-binding modes of PARP1. Analysis of the complex structure of PARP1 leads to a better understanding of the interactions between PARP1 and its inhibitors at the molecular level. This structural information will enable the design of novel PARP1 inhibitors with higher affinities.

PARP1 plays diverse and pivotal roles in many cellular signaling pathways [27–29], PARP1 inhibitors have been developed to target the active sites in the catalytic domain of PARP1. However, because drug resistance is inevitable during the development of enzyme-targeting inhibitors, including novel PARP1 inhibitors, the development of novel PARP1 inhibitors to circumvent potent drug resistance is necessary. Therefore, designing more precise inhibitors is necessary to block the functions of inhibitor-resistant PARP1 mutants. Another strategy involves the exploration of novel inhibitor-binding sites, excluding the active site of the catalytic domain.

In this review, we discuss the known structures of PARP1 and its complexes with inhibitors to elucidate the inhibitory mechanism. In addition, we summarize the profiles of currently available PARP1 inhibitors and propose therapeutic strategies for the development of novel PARP1 inhibitors, including the exploration of a possible inhibitor-binding site for PARP1. Because the development of novel PARP1 inhibitors requires profound structural insights, this review provides a comprehensive understanding of the molecular biology of PARP1, which can facilitate the development of novel anticancer agents targeting breast, ovarian, prostate, and pancreatic cancers associated with synthetic lethality.

2. Mechanisms of Action of PARPs in DNA Repair

DNA damage occurs due to various environmental factors. Generally, DNA damage is classified into four types: (1) SSBs, (2) double-stranded DNA breaks, (3) bulky adducts, and (4) base mismatches, insertions, and deletions. However, cells are equipped with a DNA damage response (DDR) system to protect themselves from risk factors. The DDR system includes four pathways: (1) base excision repair, (2) homologous recombination repair (HRR), (3) nucleotide excision repair, and (4) mismatch repair. PARPs are involved in SSB repair, and PARP1 in particular plays a central role in this process.

Binding of PARP1 to an SSB site initiates the DDR system (Figure 1a). This first step induces a structural change in PARP1, thereby activating its PARP1 catalytic function.

NAD^+ binds to the active site of PARP1 and the ADP-ribose moiety from NAD^+ is transferred to specific residues of its target proteins in a process called PARylation. PARP1 synthesizes PAR chains that serve as molecular signals to recruit a group of proteins associated with the DDR system. These proteins include DNA ligase III, DNA polymerase β, and X-ray cross-complementing gene 1 [5]. PARP1 undergoes autoPARylation, which causes a conformational change in PARP1, followed by the release of PARP1 from the SSB sites [30].

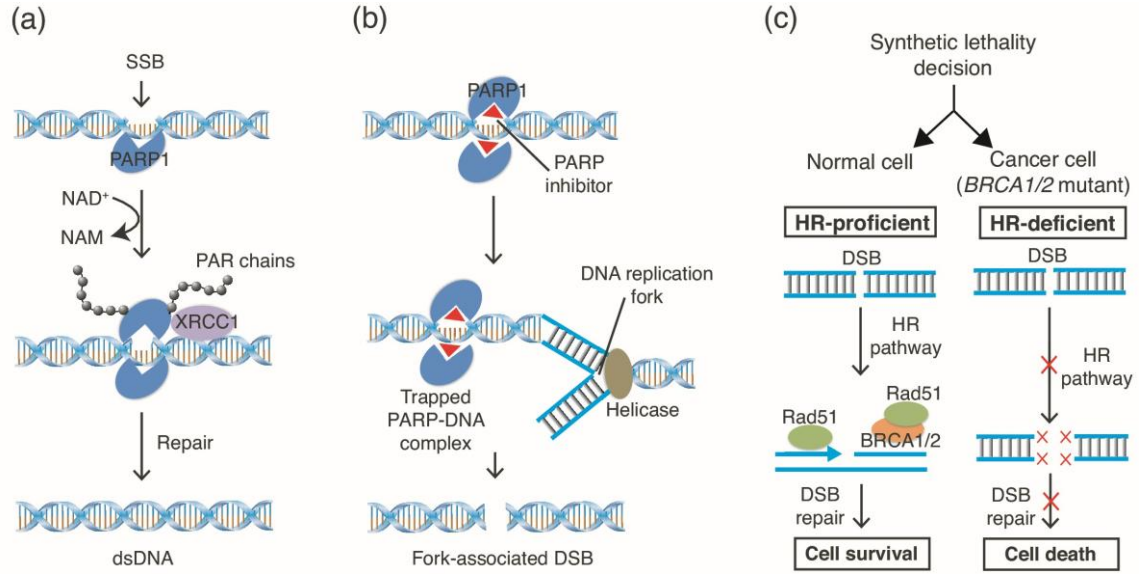


Figure 1. The biological role of PARP1 and synthetic lethality mechanism. (**a**) The role of PARP1 in repair of single-stranded breaks (SSBs) in a normal cell. (**b**) The consequence of PARP1 inhibition. (**c**) The synthetic lethality outcome in homologous recombination repair (HRR)-deficient cancer cell, compared to an HRR-proficient normal cell.

3. PARP Inhibition for Synthetic Lethality

Inhibition of PARP1 with radiotherapy allows SSBs to persist and to be converted into DSBs, ultimately leading to cancer cell death (Figure 1b). Thus, initial PARP1 inhibitors have been utilized in combination chemotherapy [31]. Subsequently, the identification of genes associated with synthetic lethality renewed interest in PARP1 inhibitors [7,8]. Studies have shown that HRR-defective cancer cells lose the ability to repair DNA breaks [7,8], suggesting that PAPR1 inhibitors can be used as single-agent therapies for HRR-defective cancers. Cancers with BRCA1/2 mutations are typical examples of HRR-defective cancers. BRCA1/2 proteins are tumor suppressors that play pivotal roles in HRR. However, mutated BRCA1/2 loses its HRR function (Figure 1c).

Mechanistically, the synthetic lethal interaction between PARP1 inhibition and BRCA1/2 deficiency extends beyond SSB accumulation. PARP inhibitors physically trap PARP1 onto DNA at damaged sites, generating highly toxic protein-DNA complexes that act as physical obstacles to advancing replication forks. This barrier forces replication, fork stalling, degradation, and subsequent collapse into lethal DSBs. Furthermore, recent studies have demonstrated that PARP1 inhibition disrupts lagging-strand maturation, resulting in the accumulation of unrepaired single-stranded DNA gaps that BRCA-deficient cells are unable to protect or restart, triggering a replication catastrophe [32–34]. BRCA1/2 mutations have been identified in familial breast and ovarian cancers [10]. Ultimately, this combined disruption of replication forks and the loss of error-free HRR drives synthetic lethality in BRCA1/2-mutated cancer cells.

4. Structural Analysis of PARP1

4.1. Primary Structure of PARP1

To date, 17 PARP enzymes (PARP1–4, -5a/b, and -6–16) have been identified [4]. Although they comprise different domains, most PARPs possess a common catalytic domain. PARP1 comprises three zinc finger (F1–3) domains at the N-terminus, a BRCA1 C-terminal (BRCT) domain, a Trp-Gly-Arg (WGR) domain, and a catalytic domain at the C-terminus (Figure 2a). The catalytic domain consists of a helical subdomain (HD) and (ADP-ribosyl)transferase (ART) domains (Figure 2a). Although the HD region is responsible for auto-inhibition, the ART domain plays a central role in catalysis because it contains an active site. In addition, the ART domain is conserved among all PARP family members. Here, we describe the tertiary structure of the catalytic domain of PARP1, excluding the F1–3, BRCT, and WGR domains.

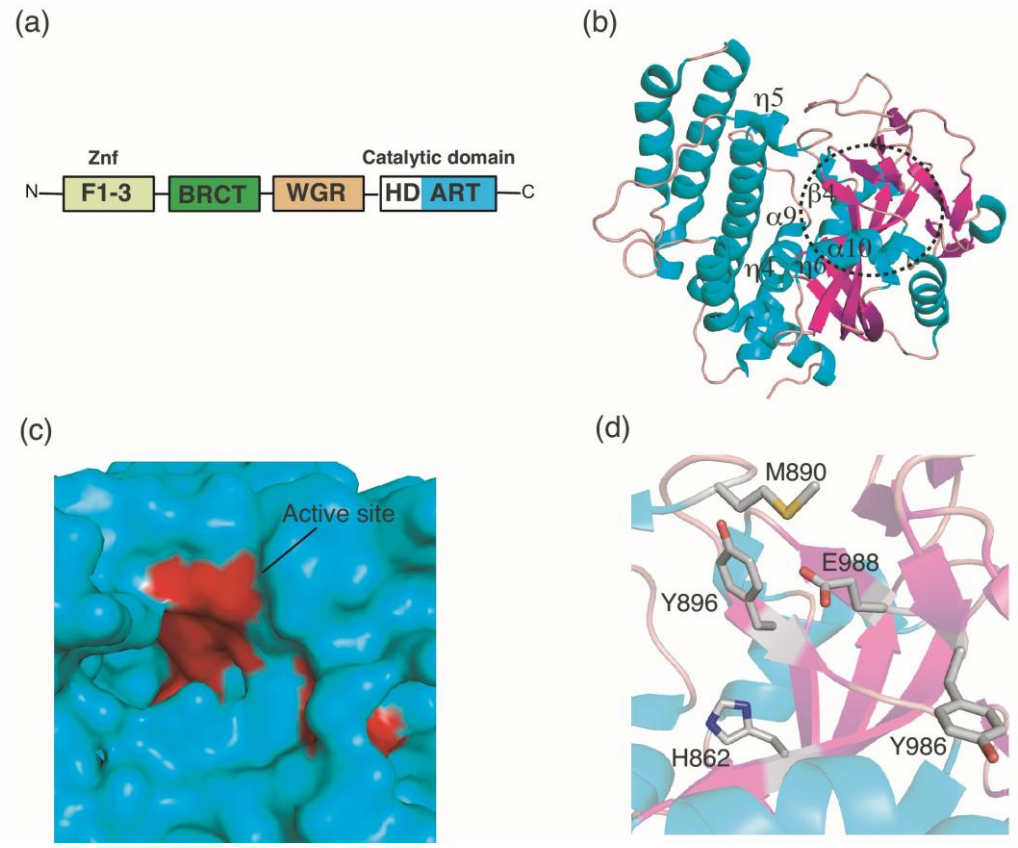
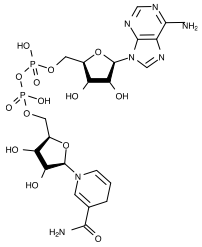
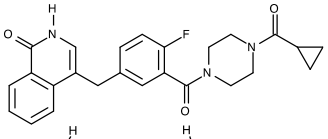
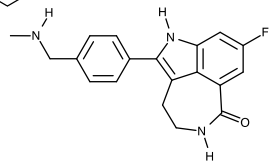
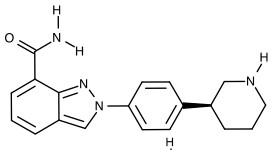
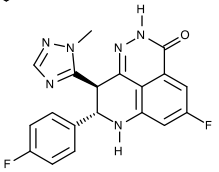
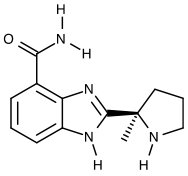
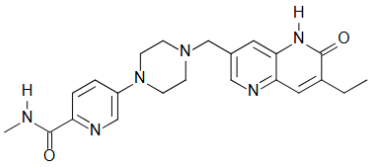


Figure 2. PARP1 structure. (**a**) The primary structure. (**b**) The tertiary structure of the catalytic domain (HD and ART). The structure is represented as cartoon. The active site is represented as surface (**c**) and cartoon (**d**). The key residues in the active site are depicted with sticks.

4.2. Catalytic Domain Structure of PARP1

Several crystal structures of the catalytic domain have been determined (Table 1). These include the structures of the catalytic domain complexed with an NAD^+ analog (PDB ID: 6BHV [21]) and its inhibitors (PDB ID: 4BJC [24], 4R6E, 4UND, 6VKK [25], 7AAC [22], 7AAD [22], 7KK3 [23], 7KK4 [23], 7KK5 [23], 7KK6 [23], and 9ETQ [26]), as well as the native catalytic domain (PDB ID: 7AAA [22] and 7KK2 [23]) (Table 1). Although the entire structure of PARP1 has not yet been elucidated, the catalytic domain structures provide valuable information for understanding its inhibitory mechanism of PARP1.

Table 1. Structural information of PARP1 complexed with its inhibitors.

PARP Family	Domain	Bound Molecule	Molecular Structure	PDB ID	Reference
1	Catalytic domain	-	-	7AAA 7KK2	[22,23]
1	Catalytic domain	BAD *		6BHV	[21]
1	Catalytic domain	Olaparib		7AAD 7KK4	[22,23]
1	Catalytic domain	Rucaparib		4BJC 6VKK	[24,25]
1	Catalytic domain	Niraparib		4R6E 7KK5	[23]
1	Catalytic domain	Talazoparib		4UND 7KK3	[23]
1	Catalytic domain	Veliparib		7AAC 7KK6	[22,23]
1	Catalytic domain	Saruparib		9ETQ	[26]

* Benzamide adenine dinucleotide (NAD^+ analog).

Elucidation of the crystal structure of the PARP1 catalytic domain in complex with an NAD^+ analog (PDB ID: 6BHV [21]), which constitutes a substituent of NAD^+ (a PARP1 substrate), enabled prediction of the substrate-binding mode. In addition, the structures of the complexes with PARP1 inhibitors were also determined. The PARP1 inhibitors in these complex structures include olaparib (PDB ID: 7AAD) [22] and 7KK4 [23], rucaparib (PDB ID: 4BJC) [24] and 6VKK [25], niraparib (PDB ID: 4R6E and 7KK5 [23]), talazoparib (PDB ID: 4UND and 7KK3 [23]), veliparib (PDB ID: 7AAC [22] and 7KK6 [23]), and saruparib (PDB ID: 9ETQ [26]). Studying how these inhibitors bind to the active site of PARP1 can provide insights into the development of novel PARP1 inhibitors. In this section, we describe the crystal structure of the catalytic domain of PARP1 in complex with the NAD^+ analog (PDB ID: 6BHV) [21].

The HD domain comprises six α-helices (α1–6) and one 3_{10}-helix (η1) (Figure 2b). The HD domain plays a role in regulating the catalytic activity of the enzyme. Specifically, in the presence of DNA, the HD domain obstructs NAD^+ binding to the active site, whereas, in the absence of DNA, this domain allows NAD^+ to access the active site. The ART domain is composed of four α-helices (α7–10), seven 3_{10}-helices (η2–8), 14 β-strands (β1–14), and several loops (Figure 2b). The active site is formed by α9, α10, η4–6, and β4 (Figure 2b,c).

4.3. Catalytic Mechanism of PARP1

Several specific residues associated with the catalytic activity are conserved in the catalytic domains of PARPs and play key roles in PARylation. These residues include His862, Met890, Tyr896, Tyr986, and Glu988 (Figure 2d). Several models have been proposed to explain this catalytic mechanism. Here, we explore a hypothetical dimeric model for catalysis. Considering that one PARP1 in the proposed dimer would function as a donor of ADP-ribose, whereas the other would serve as an acceptor, the site where ADP-ribose is attached differs depending on the type of PARylation: linear or branched. Here we describe the theoretical catalytic mechanisms of dimeric PARP1 in both forms. However, the hypothetical dimeric stoichiometry requires further experimental validation.

The first step in the proposed PARylation is the attachment of ADP-ribose to the target residue of the acceptor protein. To achieve this, donor PARP1 exploits NAD^+ as a source of ADP-ribose. Initially, NAD^+ binds to the binding pocket of the catalytic domain of the donor PARP1. His862 plays a role in fixing NAD^+ by forming a hydrogen bond between the N1 nitrogen in the imidazole ring and the -OH group linked to the 2″ carbon atom of the ribose moiety. Tyr986 forms π-π stacking with the pyridine moiety of NAM. In addition, Glu988 contributes to the simultaneous fixation of the ribose moiety linked to NAM and the target residue of the acceptor protein. The target residue is either Glu, Arg, Asp, Ser, Lys, or Cys. These residues retain a lone pair, which can attack the 1′ carbon atom. One ADP-ribose molecule is linked to the target residue via an initiation reaction.

Once an ADP-ribose molecule is attached to the acceptor PARP1, the elongation reaction proceeds in two forms: linear and branched PARylation [2]. If linear PARylation is achieved, the 2′ oxygen atom in the ribose ring linked to adenine, which is attached to the acceptor PARP1, attacks the same 1′ carbon atom in NAD^+ in the donor PARP1 as in the initiation reaction. In this case, M890 is assumed to play a vital role in fixing the adenine moiety of ADP-ribose to the acceptor PARP1. In addition, Tyr986 is associated with a pyrophosphate-binding site.

In branched PARylation, the 2′ oxygen atom in the ribose ring linked to the target residue attacks the 1′ carbon atom in NAD^+ in the donor PARP1. Thus, the difference in the initiation sites of the SN_2 reaction determines the elongation form. In contrast to linear PARylation, Met890 adopts a different orientation during branched PARylation because it does not need to interact with the adenine moiety. Although this dimeric model provides a structural rationale for both elongation forms, further biochemical validation is required to definitively confirm the existence of this dimeric state.

5. PARP1 Inhibitors and Their Binding Modes

5.1. Currently Available PARP1 Inhibitors

To date, four PARP1 inhibitors—olaparib, rucaparib, niraparib, and talazoparib—have been made available in the pharmaceutical market. Some of these drugs are currently undergoing clinical trials for novel indications.

Olaparib was first approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2014. It has been used to treat BRCA-mutated

advanced ovarian, prostate, and pancreatic cancers [12]. Olaparib was developed by KuDOS, AstraZeneca, and Merck & Co., and launched under the brand name Lynparza. Olaparib, the first PARP1 inhibitor to be used as monotherapy, targets PARP1 in patients with hereditary BRCA1/2 double mutations.

Rucaparib, sold under the trade name Rubraca, also targets PARP1. It was developed by Clovis Oncology. Rucaparib was granted accelerated approval by the FDA in 2016 to treat patients with platinum-sensitive, relapsed, high-grade epithelial ovarian cancer. Rucaparib is indicated in patients with pretreated advanced BRCA-mutated ovarian and prostate cancers [14]. It was approved by the EMA in 2018.

Niraparib, which is sold under the brand name Zejula, was developed by Tesaro. The major targets of niraparib are PARP1 and PARP2. This drug was approved for the treatment of epithelial ovarian, fallopian tube, and primary peritoneal cancers by the US FDA in 2017 and by the EMA in the same year [17].

Talazoparib (brand name Talzenna) is marketed by Pfizer. It has applications in the treatment of advanced breast and prostate cancers with germline BRCA mutations and HER2-negative, locally advanced, or metastatic breast cancers [19,20]. The US FDA and EMA granted approval in 2018 and 2019, respectively. Talazoparib is also known to trap PARP, which means that PARP1 is stabilized by being trapped on bound DNA, eventually leading to DNA damage [16,35].

Lastly, saruparib, developed by AstraZeneca, is an investigational selective PARP inhibitor. The main target of saruparib is PARP1, which exhibits an IC_{50} value of 3 nM [36,37]. This inhibitor traps PARP1 on SSBs [38]. Recently, saruparib is undergoing Phase 3 clinical trials to test for advanced breast cancer with *BRCA* or *PALB2* mutations, and metastatic hormone-sensitive prostate cancer [39].

5.2. Structures of PARP1-Inhibitor Complexes

The catalytic domain of PARPs has an NAD^+-binding pocket from which ADP-ribose is transferred to a specific residue in the target protein. PARPs with PARylation activity (PARP1–5) retain an NAD^+ binding site in their catalytic domain. Specifically, the NAD^+ binding site contains a conserved His-Tyr-Glu (HYE) triad. His and Tyr residues in this triad are responsible for binding to an NAD^+ molecule, whereas the Glu residue plays a role in fixing the NAD^+ molecule and the target residue. Such information on the interactions between key residues and substrates provides structural insights into inhibitor-binding modes for designing PARP inhibitors.

As described in Section 5.1, several PARP1 inhibitors have been approved for clinical use. Accordingly, the crystal structures of PARP1-inhibitor complexes, including those of olaparib (PDB ID: 7AAD [22] and 7KK4 [23]), rucaparib (PDB ID: 4BJC [24] and 6VKK [25]), niraparib (PDB ID: 4R6E and 7KK5 [23]), talazoparib (PDB ID: 4UND and 7KK3 [23]), and saruparib (PDB ID: 9TEQ [26]) have also been determined. The crystal structure of the PARP1-NAD^+ analog has been reported as well (PDB ID: 6BHV [21]).

In the next section, we describe the crystal structure of the PARP1-NAD^+ analog and the structures of PARP1 in complex with its inhibitors.

5.2.1. Binding Mode of the PARP1-NAD^+ Analog Complex

Benzamide adenine dinucleotide (BAD) is an NAD^+ analog containing a benzene ring instead of a pyridine ring in the NAM moiety (Table 1). Thus, the carbon atom in BAD is substituted for the nitrogen atom in NAD^+. The structure of PARP1 in complex with BAD exhibits a virtual substrate-binding mode (Figure 3a). In the BAD complex structure, many residues in the NAD^+-binding site interact with BAD (Figure 3b). Specifically, His862 and Ser864 form hydrogen bonds with the oxygen atom in adenosine ribose. Gly876 and Arg878 also interact with the nitrogen atoms in the adenine moiety. Tyr889

and Met890 interact with the phosphate group in adenosine and the ribose ring in the NAM nucleotide, respectively. In addition, Gly863, Ala880, Gly888, Gly894, and Tyr896 interact with BAD, mediated by inner water molecules (Figure 3b). Hydrophobic interactions are also found during BAD binding (Figure 3b). The residues associated with these interactions include Asn868, Gly871, Ile872, Gln875, Leu877, Phe897, Tyr907, and Glu988.

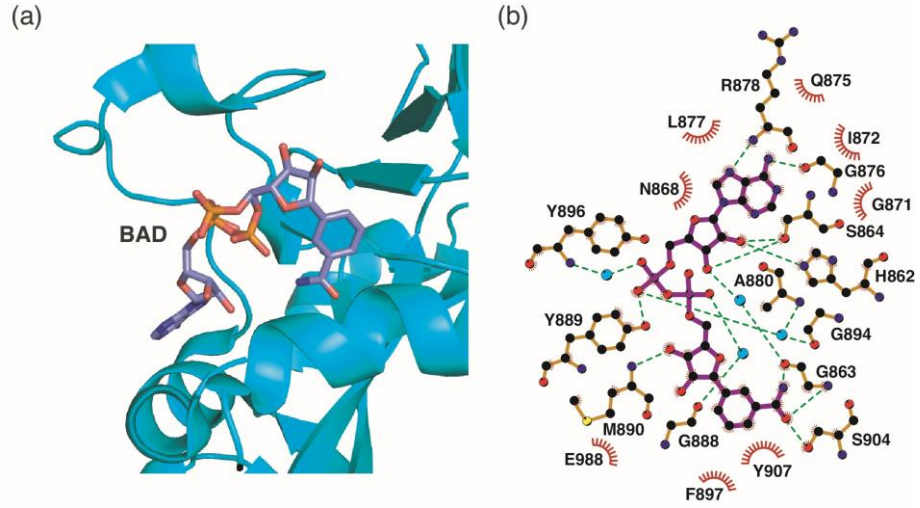


Figure 3. NAD⁺ analog-binding mode. (**a**) Crystal structure of the active site in the catalytic domain in complex with NAD⁺ analogue (benzamide adenine dinucleotide; BAD). The protein and BAD structures are represented as ribbon and sticks, respectively. (**b**) Schematic diagram of BAD showing interactions with adjacent residues. The cyan circles and green dashed lines indicate water molecules and hydrogen bonds, respectively.

5.2.2. Binding Mode of the PARP1-Olaparib Complex

Olaparib was the first PARP1 inhibitor approved for clinical use. The structure of PARP1 in complex with olaparib shows its binding mode (Figure 4a). The phthalazine moiety of olaparib interacts with Gly863 and Ser904 by forming hydrogen bonds (Figure 4b). Specifically, the backbone N-H hydrogen atom of Gly863 and the hydrogen in the side chain of Ser904 simultaneously form hydrogen bonds with the oxygen atom linked to the phthalazine ring. In addition, the backbone N-H hydrogen atom of Tyr896 forms a hydrogen bond with the oxygen atom in the carbonyl group linked to the piperazine ring (Figure 4b). Arg878, Ala880, and Gly894 were also found to interact with olaparib in a reaction mediated by adjacent water molecules (Figure 4b). Additionally, olaparib hydrophobically interacts with peripheral residues, such as Asp766, Leu769, Asp770, His862, Tyr889, Ile895, Phe897, Ala898, Tyr907, and Glu988 (Figure 4b).

This interaction analysis suggests a rationale for the lack of isoform selectivity of first-generation PARP inhibitors. The core hydrogen bonds associated with Gly863, Ser904, and Tyr896 target the highly conserved nicotinamide-binding pocket, which is structurally highly similar in both PARP1 and PARP2. This structural mimicry partially accounts for the dual-inhibition profile that has been associated with clinical hematological toxicities. However, designing next-generation PARP inhibitors could potentially exploit adjacent residues like Tyr907, which represent a key evolutionary difference between PARP1 and PARP2. While PARP1 possesses a hydrophobic sub-pocket known as the "TY pocket" formed by this flexible Tyr907 residue, PARP2 retains a rigid cysteine residue (Cys983) at the equivalent position. Consequently, modifying chemical moieties to sterically exploit the spatial flexibility around Tyr907 may represent a viable strategy for achieving high PARP1 selectivity, thereby potentially minimizing off-target toxicities.

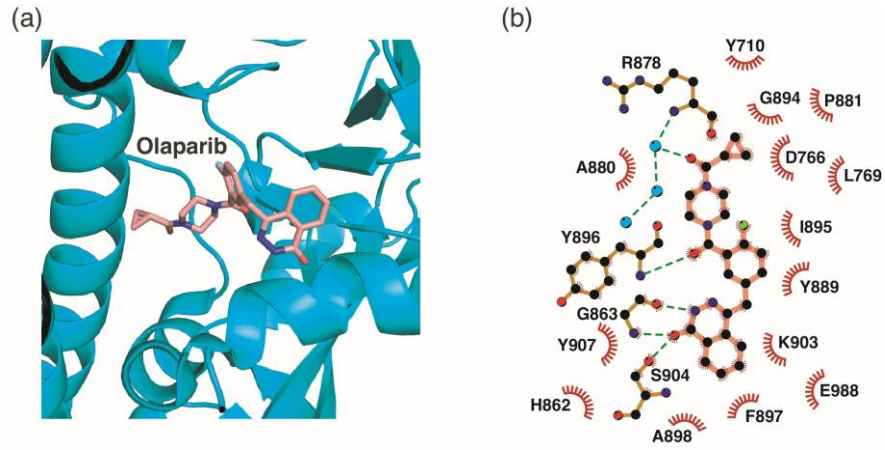


Figure 4. Olaparib-binding mode. (**a**) Crystal structure of the catalytic domain in complex with olaparib. (**b**) Schematic diagram of olaparib showing interactions with adjacent residues.

5.2.3. Binding Mode of the PARP1-Rucaparib Complex

Rucaparib was the second PARP1 inhibitor to be approved for clinical use. The structure of PARP1 in complex with rucaparib also exhibits its binding mode (Figure 5a). In contrast to the olaparib-PARP1 structure, rucaparib interacts with fewer residues. The hydrogen atom linked to N3 forms a hydrogen bond with Glu763 (Figure 5b). In addition, the hydrogen atom linked to N1 and the oxygen atom linked to C7 form hydrogen bonds with the backbone atoms of Gly863 and the hydrogen atom in the side chain of Ser904 (Figure 5b). Glu988 also interacts with the hydrogen atom linked to N2 via water molecules. Similar to olaparib, rucaparib interacts hydrophobically with adjacent residues, including Asp766, His862, Gly888, Tyr889, Tyr896, Phe897, Ala898, and Tyr907 (Figure 5b).

This binding mode highlights potential core elements for competitive PARP inhibitor design. Specifically, rucaparib is characterized by a flat tricyclic azepinoindole core, which differs from the bulky phthalazine-piperazine scaffold in olaparib. Another important structural feature is the direct hydrogen bond with Glu763 in the regulatory HD region. This interaction is proposed to stabilize the catalytic domain in a rigid conformation, potentially explaining the distinct DNA-trapping ability of rucaparib despite its interactions with fewer residues than olaparib. However, because both inhibitors interact with conserved hydrophobic residues, such as Tyr889, Tyr896, and Tyr907, rather than isoform-specific pockets, this interaction profile likely contributes to dual PARP1/PARP2 inhibition.

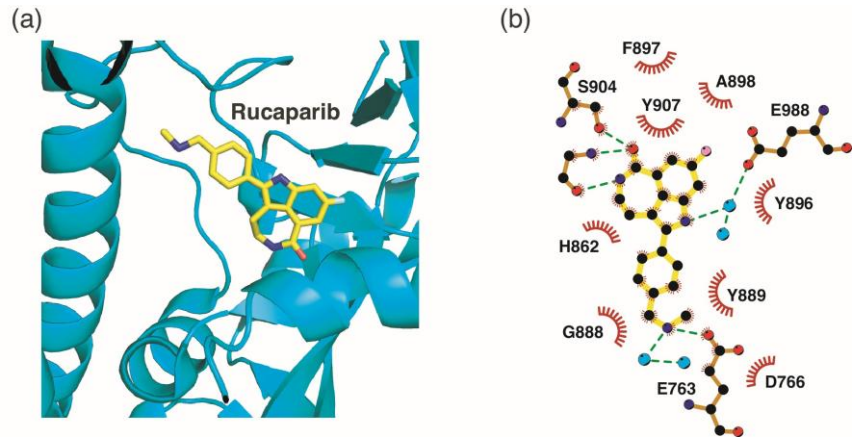


Figure 5. Rucaparib-binding mode. (**a**) Crystal structure of the catalytic domain in complex with rucaparib. (**b**) Schematic diagram of rucaparib showing interactions with adjacent residues.

https://doi.org/10.3390/biom16111603

5.2.4. Binding Mode of the PARP1-Niraparib Complex

Niraparib was the third PARP1 inhibitor to be approved for clinical use. The structure of PARP1 in complex with niraparib provides molecular information on its binding mode (Figure 6a). The nitrogen atom in the piperidine ring interacts with Gln759 and Thr887 via a water molecule (Figure 6b). Additionally, the amide group linked to the indazole ring directly forms hydrogen bonds with Gly863 and Ser904. Compared to olaparib and rucaparib, niraparib exploits more hydrophobic interactions. The residues associated with these hydrophobic interactions include Val762, Asp766, His862, Gly888, Tyr889, Tyr896, Phe897, Ala898, Lys903, Tyr907, and Glu988 (Figure 6b).

These extensive hydrophobic interactions may provide useful structural insights for optimizing PARP inhibitors. Because of its flexible piperidine-phenyl rotatable core, niraparib achieves spatial packing against Val762 and Lys903. This tight hydrophobic network, along with the core hydrogen bonds, is expected to contribute to the formation of a highly stable complex, potentially explaining its potent DNA-trapping activity. Furthermore, the minimization of highly polar groups enhances lipophilicity. Consequently, this structural feature partially explains the higher cell permeability and blood–brain barrier penetration of niraparib compared with olaparib or rucaparib.

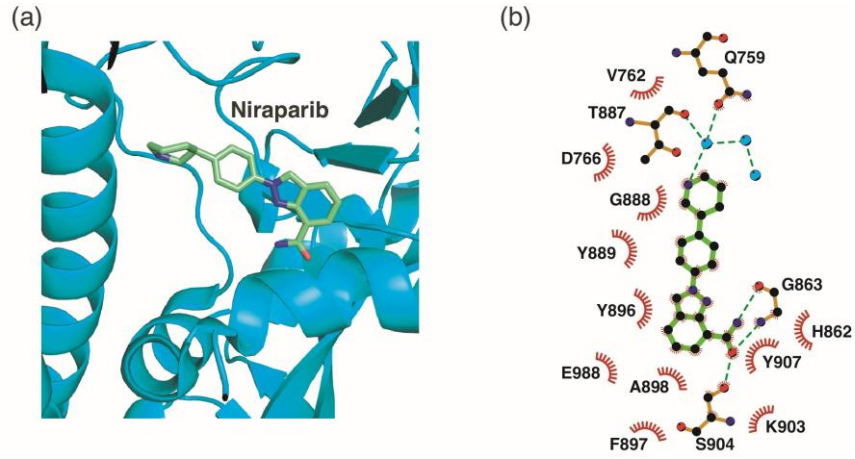


Figure 6. Niraparib-binding mode. (**a**) Crystal structure of the catalytic domain in complex with niraparib. (**b**) Schematic diagram of niraparib showing interactions with adjacent residues.

5.2.5. Binding Mode of the PARP1-Talazoparib Complex

Talazoparib was the fourth PARP1 inhibitor approved for clinical use. The structure of PARP1 in complex with talazoparib also provides specific information on its binding mode (Figure 7a). In contrast to other PARP1 inhibitors, talazoparin contains many nitrogen atoms, which allows it to form adequate hydrogen bonds. Specifically, the phthalazine moiety forms hydrogen bonds with Gly863 and Ser904, whereas the nitrogen atom of the pyridine moiety interacts with Glu988 via a water molecule (Figure 7b). In addition, the N4 nitrogen atom in the triazole ring interacts with Tyr896 also via a water molecule (Figure 7b). In addition, talazoparib hydrophobically interacts with neighboring residues such as His862, Gly888, Tyr889, and Tyr907 (Figure 7b).

This structural analysis suggests a rationale for the exceptionally high clinical potency of talazoparib as a first generation DNA-trapping PARP inhibitor. In contrast to the smaller scaffolds of other PARP inhibitors, talazoparib possesses a highly rigid multi-ring system capable of filling the active site pocket. The abundant nitrogen atoms are positioned to form a water-mediated hydrogen bond network, stabilizing the drug in the active site. This structural feature is proposed to create substantial steric hindrance that locks the enzyme into an inactive conformation. Such conformational fixation likely prevents

the structural rearrangements necessary for PARP1 to release itself from DNA, thereby contributing to its high trapping efficiency.

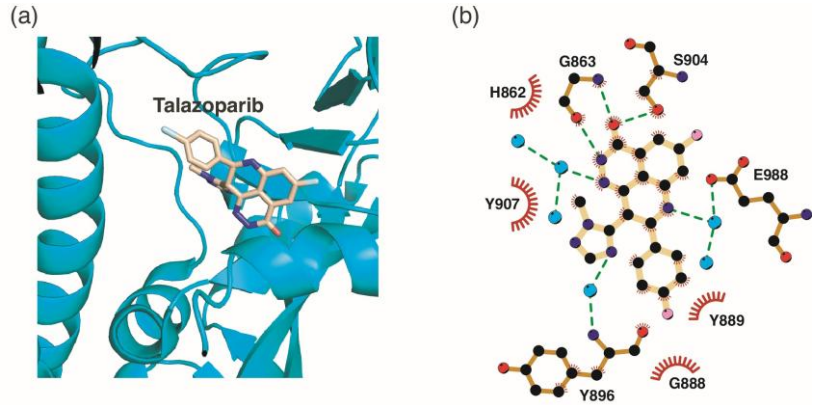


Figure 7. Talazoparib-binding mode. (**a**) Crystal structure of the catalytic domain in complex with talazoparib. (**b**) Schematic diagram of talazoparib showing interactions with adjacent residues.

5.2.6. Binding Mode of the PARP1-Saruparib Complex

Saruparib is an investigational next-generation selective PARP1 inhibitor undergoing Phase 3 clinical trial. The structure of PARP1 in complex with saruparib provides molecular information on its binding mode (Figure 8a). Saruparib is characterized by naphthyridine, piperazine, and pyridine rings containing several nitrogen atoms that form hydrogen bonds. Specifically, the naphthyridine moiety forms hydrogen bonds with Gly863 and Ser904, and interacts with Tyr907 through π-π stacking. The terminal methylcarboxamide forms a hydrogen bond with Leu879 (Figure 8b). As other PARP inhibitors, saruparib also hydrophobically interacts with adjacent residues, such as Leu769, His869, Arg878, Pro881, Tyr896, Ala898, and Tyr907 (Figure 7b).

The molecular structure of saruparib represents a promising strategy for transitioning from non-selective dual PARP1/PARP2 inhibition. Saruparib forms hydrogen bonds with Gly863 and Ser904, which are though to facilitate stable binding in the catalytic nicotinamide pocket. Notably, the molecular backbone extends directly toward Tyr907, potentially exploiting the flexibility of this residue in PARP1. Because PARP2 possesses a rigid Cys983 residue at the equivalent position, saruparib is predicted to sterically clash with the corresponding residue, owing to the spatial configuration of the active site in PARP2. Such a structural difference likely underlies its reported 500-fold selectivity for PARP1 [37]. Furthermore, the terminal methylcarboxamide moiety interacts with Leu769 outside the active site, potentially stabilizing the PARP1-saruparib complex. These structural features offer a compelling rationale for its high selectivity for PARP1 over PARP2.

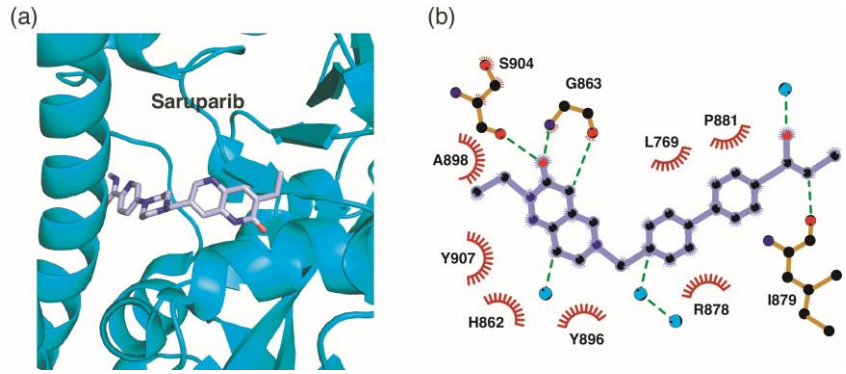


Figure 8. Saruparib-binding mode. (**a**) Crystal structure of the catalytic domain in complex with saruparib. (**b**) Schematic diagram of saruparib showing interactions with adjacent residues.

6. Mechanisms of Resistance to PARP Inhibitors

There are several reported cases of reversion mutations in BRCA1/2 acquiring resistance to PARP inhibitors and platinum-based anticancer agents [40]. The fundamental molecular mechanism is that secondary mutations such as base substitutions, insertions, and deletions restore the original wild-type open reading frame of BRCA1/2. Such a reversion to normal BRCA1/2 genes results in the restoration of HRR function. These molecular events lead to acquired resistance to PARP inhibitors, rendering them ineffective. In addition to genetic reversions, tumors can functionally restore HRR through the loss of end-protection factors. Specifically, somatic loss of 53BP1 or components of the downstream Shieldin complex alleviates the block on DNA end resection in BRCA1-deficient cells [41,42]. This loss permits alternative path activation to process double-strand breaks, thereby driving PARP inhibitor resistance without modifying the original BRCA1 mutation [41,42].

Furthermore, cancer cells can develop resistance without restoring HRR simply by protecting and stabilizing stalled replication forks. In BRCA-deficient cancer cells, replication forks are vulnerable to catastrophic degradation by nucleases like MRE11 [43]. However, resistant cancer cells bypass this by overexpressing fork-protective proteins such as FANCD2 or by losing destabilizing factors such as PTIP or SMARCAL1 [44,45]. This shields the nascent DNA strands from degradation and maintains an active replication process under PARP-mediated stress.

Another recent study showed that catalytic domain mutations in PARP1 cause significant resistance to saruparib, which is an investigational PARP1 inhibitor, in multiple phase 3 clinical trials [46]. The authors of the study established five saruparib-resistant cell lines through high-level dosing, similar to clinical exposure. All these mutations produced catalytic domain mutations in PARP1, which exhibited high resistance to saruparib. These results demonstrate that key residue mutations in the active site reduce the PARP1 inhibitor-binding affinity. Notably, these active-site mutations alter PARP trapping in a unique manner, allowing first-generation dual inhibitors to work against saruparib-resistant cells.

Lastly, the upregulation of drug efflux pumps provides resistance against PARP inhibitors. For example, P-glycoprotein 1 (P-gp) ubiquitously expressed in several organs and is known as a multidrug resistance protein 1, exports various exogenic molecules. Thus, this membrane protein plays a critical role in decreasing drug efficacy by transporting drug molecules out of cells. Several cancer cells overexpress P-gp and exhibit strong multidrug resistance. Preclinical studies have shown that overexpression of P-gp in BRCA-deficient mouse models results in olaparib resistance [47]. Previous studies have demonstrated that P-gp overexpression in ovarian cancer cells reduces the efficacy of talazoparib [48]. Therefore, the inhibition of P-gp function may be a successful strategy to overcome PARP inhibitor resistance. Combination therapy with PARP and P-gp inhibitors can be considered a clinical approach to maintain the therapeutic level of PARP inhibitors.

7. Drug Design Strategies for the Development of Novel PARP1 Inhibitors

Resistance to inhibitors naturally occurs in drug-targeted enzymes, such as kinases [49,50]. Specifically, mutations in key residues in the active site are frequently observed over time in cancer cells and pathogenic bacteria treated with long-term therapies such as anticancer agents and antibiotics. Mutations in cancer cells and pathogenic bacteria reduce the affinity of inhibitors for the binding residues. These phenomena necessitate the development of novel inhibitors to overcome anticancer and antibiotic resistance [49,50].

Similar to other enzyme inhibitors, the development of novel PARP1 inhibitors requires an increase in the affinity for specific binding sites. In PARP1, the known inhibitor-binding site is the NAD^+-binding site. Two molecular approaches could be used to design novel PARP1 inhibitors. The first involves modifying the structures of known inhibitors. As discussed in Section 5.2, we already have specific information about the molecular structures and binding modes of the currently available PARP1 inhibitors. Each PARP1 inhibitor binds specifically to several residues in its binding site. Based on the accumulated information on the interactions of inhibitors with their binding residues, several pharmacophore features of PARP1 inhibitors can be elucidated, enabling pharmacophore-based inhibitor design. These features provide information about the essential chemical groups that properly bind to the corresponding residues. Additionally, if specific residues that bind NAD^+ have critical effects on the inhibitor-binding affinity, a novel moiety that binds to the residues associated with NAD^+ binding should be considered when designing novel inhibitors. This strategy involves the addition of new hydrogen bonds, ion-ion interactions, dipole–dipole interactions, ion–dipole interactions, and hydrophobic interactions.

Another approach to designing novel PARP1 inhibitors is to mimic the molecular structure of NAD^+. Because NAD^+ is a natural substrate of PARP1, it is reasonable to assume that PARP1 has significant binding affinity for NAD^+. Thus, it may be an appropriate starting molecule for designing novel PARP1 inhibitors. As described in Section 5.2.1, several residues at the NAD^+ binding site are involved in NAD^+ binding. This information provides structural insights for the design of NAD^+-based inhibitors. Similar to the ATP-based design of kinase inhibitors, many other inhibitors have been designed using information on the surrounding residues in the ATP-binding pocket. At the NAD^+-binding site, NAD^+ coordinates with the surrounding residues. Therefore, key interactions between NAD^+ and the surrounding residues should be considered in the design of PARP1 inhibitors. In addition, the NAM moiety, including the covalent bond with the neighboring ribose, should be excluded when designing PARP1 inhibitors, because an aspartate residue or a conjugated NAD^+ in the counterpart PARP1 as a nucleophile attacks this covalent bond site in the SN_2 reaction mechanism.

Lastly, the inhibition of PARP1 dimerization may be another strategy. Although researchers have debated whether PARP1 functions as a monomer or dimer, many previous studies have shown that PARP1 plays a biological role as a dimer [51–53]. Thus, if this stoichiometric property is critical for the function of PARP1, blocking the dimerization of PARP1 may effectively inhibit PARP1 activity. However, this strategy requires a comprehensive understanding of PARP1 dimerization.

Dimerization of PARP1 has been proposed to yield symmetric and asymmetric homodimers [2]. It can facilitate intermolecular *trans*-activation, although recent studies also support an allosteric *cis*-activation mechanism, in which it functions as a monomer [54,55]. In the hypothesized symmetric homodimer, the two monomers simultaneously function as both the donor and acceptor of PARP1 for PARylation. However, in the asymmetric homodimer, one monomer functions as a donor for PARylation and the other constitutes an acceptor. Accordingly, identification of spatial clefts at the interface between the two monomers could yield candidate target sites for inhibition of PARP1 function. In future studies, it will be necessary to clarify the working mode of PARP1 in dimerization and to unveil the interface structure. These findings may facilitate the development of novel PARP1 inhibitors.

8. Discussion

PARP1 is a central enzyme in PARylation and is associated with vital cellular maintenance processes such as DNA repair [27–29]. The basic concept behind targeting PARP1

during chemotherapy with anticancer agents is to block its function of PARP1, thereby preventing DNA repair and inducing cell death. The catalytic domain, including the ART domain, is considered a key site for the binding of PARP1 inhibitors because it contains an NAD^+ binding site. Accordingly, a comprehensive understanding of the ART domain structure and function may lead to the development of effective PARP1 inhibitors. However, as with other drug-targeting enzymes, mutations in the active site of PARP1 are expected to occur at the inhibitor-binding site over time. These mutations can lead to the development of resistance to existing inhibitors. Therefore, novel PARP1 inhibitors need to be developed.

Additionally, various combination therapies can serve as alternatives to overcome resistance to PARP inhibitors [56]. For example, combination therapies of PARP inhibitors with ataxia telangiectasia and Rad3-related protein (ATR) inhibitors or WEE1 G2 checkpoint kinase (WEE1) inhibitors have been actively studied in preclinical and early phase clinical trials to overcome acquired resistance to PARP inhibitors [56,57]. Malignant cells frequently develop resistance to PARP inhibitors by restoring HR repair or stabilizing replication forks [57]. However, inhibitors targeting ATR or WEE1 disrupt the specific molecular mechanisms [57,58]. By increasing DNA replication stress, these drug combinations induce a functional HR-deficient state that causes premature entry into mitosis, resulting in mitotic collapse and cell death [58]. Recent clinical trials have demonstrated that this approach can reduce treatment resistance in biomarker-selected populations, including patients with triple-negative breast cancer, metastatic prostate cancer, and high-grade serous ovarian cancer [56,57]. Specifically, clinical evaluations of ATR inhibitors, such as ceralasertib, combined with olaparib in the CAPRI and OLAPCO studies have demonstrated encouraging therapeutic activity in patients who progressed with prior PARP inhibitor regimens [56,57]. Clinical studies of WEE1 inhibitors, such as adavosertib combined with olaparib in the EFFORT trial, have also shown comparable clinical activity [56,57].

Previous structural studies of PARP1 have provided insights into its mechanism of PARP1 [21–25]. Several crystal structures of PARP1 in complex with its inhibitors and NAD^+ analogs have been reported [21–25]. This structural information has revealed how key residues in the active site of PARP1 interact with small molecules. Such binding modes suggest that compounds with stronger bonds to key residues could constitute novel PARP1 inhibitors. In addition, the binding mode of talazoparib involves the "trapping effect." [16,35]. Therefore, a molecular understanding of binding modes at the active site is critical for designing novel PARP1 inhibitors.

The primary clinical limitation of first-generation PARP inhibitors such as olaparib, rucaparib, niraparib, and talazoparib is hematological toxicity, manifested as anemia, neutropenia, and thrombocytopenia [56,59]. Preclinical and clinical studies indicate that, while synthetic lethality in HR-deficient cancers is induced mainly by the inhibition of PARP1, the simultaneous inhibition of PARP2 in hematopoietic stem and progenitor cells accounts for a significant portion of this myelosuppressive toxicity [56,59]. Thus, first-generation dual inhibitors require careful reevaluation because their lack of selectivity may compromise patient compliance and safety in both single-agent and combination therapies [56,59].

Subtle structural differences exist between the catalytic domains of PARP1 and PARP2, despite their high evolutionary conservation [56,59]. The active site structures of PARP1 and PARP2 are nearly identical in shape and residue composition [56]. This structural similarity enables first-generation PARP inhibitors with traditional nicotinamide-mimicking pharmacophores to bind PARP1 and PARP2 with nearly equivalent affinity [56,59]. Next-generation PARP1 inhibitors with high selectivity exploit subtle but significant structural features within the active site architecture [56,59]. Specifically, PARP1

possesses Tyr907 in its active site, whereas Cys983 is positioned at an equivalent position in PARP2 [59]. This molecular difference creates a deeper and more hydrophobic subpocket in PARP1 that can be sterically targeted by next-generation selective PARP1 inhibitors such as saruparib [56,59].

It remains unclear whether PARP1 functions as a monomer or dimer at the cellular level. Nevertheless, a wealth of biochemical data has demonstrated that PARP1 acts as a dimer, at least at the molecular level [51–53]. If this stoichiometric rule is applied at the cellular level, blocking PARP1 dimerization could be an innovative strategy for the development of novel PARP1 inhibitors. Because PARP1 comprises several domains, diverse combinations are possible. Therefore, it is necessary to identify the interacting domains. Once the interface architecture of a dimer is elucidated, disrupting the dimerization of the two monomers may inhibit autoPARylation.

In other enzymes, several monomer–monomer interfaces have been targeted to disrupt dimerization and inhibit enzymatic activity. For example, human thymidylate synthase, which plays a vital role in DNA synthesis, functions in its homodimeric form. A previous study demonstrated that artificially designed small molecules bind to the dimer interface, thereby rendering thymidylate synthase inactive [60]. In this study, several compounds were found to inhibit the enzymatic activity and cell growth of human pancreatic and ovarian cancer cells [60]. In another study, interference with the dimerization of SARS-CoV-2 main protease was reported [61]. This protease functions as a homodimer, and is involved in viral replication. The authors designed a small computationally engineered protein to disrupt dimerization of this protease; the results demonstrated that small proteins bound to the interface inhibited the catalytic activity of the protease [61]. Considering the possibility of PARP1 dimerization for its enzymatic function, future studies should focus on identifying the interaction sites for the dimerization of PARP1.

Recently, attention has been directed toward the dynamic properties of biomolecules, including proteins, to gain structural insights—in particular, appropriate conformers that interact with partner molecules [62]. Thus, we can obtain structural information on the specific conformers of PARP1 upon binding its substrate and inhibitors, as well as during the formation of dimers. The next step in PARP research should be a more focused investigation of the dynamic behavior of PARP1. Moreover, the stoichiometric properties of PARP1 in solution must be elucidated. As described above, it remains unclear whether PARP1 acts as a monomer or dimer during PARylation at the cellular level. Cryogenic electron microscopy (cryo-EM) and high-speed atomic force microscopy (HS-AFM) may be useful tools for studying this mechanism. Cryo-EM and HS-AFM can be used to show different conformations of PARP1 in solution. Consequently, understanding the dynamic behaviors of PARP1 may enable the precise design of novel PARP1 inhibitors with geometrically suitable binding sites. In addition, these approaches may provide structural information for the identification of novel interface sites upon dimer formation. A comprehensive understanding of the molecular biology of PARP1 will yield a new paradigm for the development of PARP1 inhibitors.

9. Conclusions

The elucidation of the structural and functional properties of PARP enzymes is essential for the rational design of novel anticancer agents targeting synthetic lethal malignancies. Through the use of first-generation PARP inhibitors, researchers have successfully exploited synthetic lethality in HR-deficient cancers; however, the dual activity of these inhibitors with PARP2 within the highly conserved NAD^+-binding site is known to cause hematological toxicity. Next-generation selective PARP1 inhibitors, such as saruparib, successfully eliminate off-target toxicities by targeting the unique "TY pocket." This strategy also circumvents emergent mechanisms of drug resistance. Determining the

multimeric state of PARP1 in the cellular environment is critical. Multifaceted analyses using advanced biophysical tools will reveal its intrinsic dynamic behavior. In conclusion, a comprehensive understanding of PARP enzymes will lead to the development of next-generation highly selective PARP1 inhibitors.

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